LABORATORY METHODS FOR BEAN QUALITY EVALUATION

A BEAN NETWORK REPORT PREPARED FOR
THE INTERNATIONAL DEVELOPMENT RESEARCH CENTRE
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CONTENTS

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INSTRON (OTMS) PEAK FORCE MEASUREMENT OF COOKED BEANS

Purpose: Determination of the peak force (expressed as newtons) to compress and extrude a representative sample of cooked beans. Used to indicate the hardness or doneness of the cooked sample.

Scope: Applicable to all types of beans, cooked for varying lengths of time.

Limitations: Composite sample of many beans tested, therefore, individual differences among beans unknown; the range of hardness among individual beans is not evident from the value obtained for the composite sample.

Apparatus:

1. Household stove or Labconco Crude Fiber Testing Apparatus (Labconco Corp., Kansas City, Missouri)
2. Instron Universal Texture Testing System (Instron Canada, Burlington, Ontario) [or Ottawa Texture Measuring System (Canner's Machinery Ltd., Simcoe, Ontario)]. Data are recorded using an Apple 2e computer, connected to the texture testing instrument.
3. 8-Bar Wire Extrusion Cell, 10 cm2 (Model D-1203, Canner's Machinery Ltd., Simcoe, Ontario). This cell is composed of a box cell with a removable wire grid insert. A chamfered square plate attached to a shaft make up the piston that compresses, shears and extrudes the sample through the grid.
4. Texture Data Acquisition and Analysis Computer Program (Agriculture Canada, Ottawa, Ontario, 1986)
5. Analytical balance, accurate to 0.01g
6. Water bath with lid
7. Metal or plastic sieve
8. Pyrex pots with lids, or beakers with covers for cooking on household stove
9. Assorted beakers and containers with lids for soaking and storage of beans.

Sample Preparation:

1. Household Stove - Weigh approximately 30-35g beans, rinse gently with warm water and soak in a 5:1 distilled water/bean ratio for 24 hr in a covered 25°C water bath. Drain the soaked beans for 2 min in a sieve and place in a pot or beaker with distilled water, using a 5:1 ratio of water to unsoaked bean weight. Quickly bring the beans to a boil on high heat in an uncovered pot. Once the water is boiling, reduce the temperature to maintain a slow boil and cover the pot. Begin cooking time measurement when the water starts to boil. Maintain the water at a

constant level throughout cooking by adding boiling water. Cook 2 hr, drain the beans for 2 min, then put them in a covered container and cool for 1 hr at room temperature (21°C).

2. Crude Fiber Testing Apparatus - This apparatus consists of six heaters with cold water canopy condensers and six 600 ml beakers that fit snugly under the condensers.

   Soak approximately 30g beans as described above and place in 600 ml beakers with distilled water, using a 5:1 ratio of water to unsoaked bean weight. Quickly bring the beans to a boil on high heat and then reduce the temperature of the heater to maintain a slow boil. Begin cooking time measurement when the water starts to boil. The water level is maintained by water condensation. Cook the beans 2 hr, drain and cool as described above.

Procedure:

The hardness of the cooked beans is measured on an Instron Universal Texture Testing System using a 10 cm^2, 8 bar, wire extrusion cell and a 90.8 kg (200 lb) load cell. Test conditions include a crosshead speed of 10 cm/min, a test time of 120 sec and a transducer capacity of 90.8 kg. The piston used to compress and extrude the sample is returned to its starting position automatically when it reaches a point 2.5 mm from the bottom grid.

Attach the signal conditioner to the Instron. The signal conditioner should be turned on at least one half hour before use to allow it to warm up. Mount the load cell on the moving crosshead of the Instron and attach the chuck and piston. Calibrate the Instron-Apple 2e system using weights or a Digital Force Calibration Gauge (Ametek Inc., Hatfield, Penn.). Attach the extrusion cell box to the drip plate of the Instron in preparation for testing.

Place a 30g. sample of cooked beans in the extrusion cell and distribute the sample evenly by lightly knocking the cell upon a padded surface five times. Record peak force to extrude in newtons (N).

Determine peak force values on duplicate aliquots of the cooked beans and average the two readings.

Replicate the test.

Determine the mean and standard deviation.

Notes:

All samples must be cooled for the same length of time (ie. to the same temperature) before the extrusion test is performed. Sample texture changes as temperature decreases and moisture is lost. Voisey and Larmond (1971a) found that the toughness of soy beans increased with time after cooking. The change was very rapid in the first hour (0.5 kg/min). Therefore, samples should be cooled for at least 1 hr to minimize this effect, and the length of the cooling period should be carefully controlled.

The quantity of sample used in this test may differ. Sefa-Dedeh et al. (1978, 1979) used 48g cooked beans per determination. Voisey and Nonnecke (1973) reported that it was not necessary to weigh a sample of cooked peas for testing as filling the cell to capacity controlled the sample size adequately. This may be true when testing one type of bean, however, if testing a number of different lines of beans, weighing the samples may be more appropriate. Voisey and Larmond (1971b) have shown with baked beans that the actual size of the sample used is not critical, as long as it is consistent.

Variations to this method have been reported. Plhak et al. (1987) soaked beans for 18 hr at 20°C and reported the peak force as kg/30g cooked beans. Hohlberg and Stanley (1987) cooked beans in a 4:1 ratio of water to beans. Hincks et al. (1987) soaked the beans for 18 hr at 25°C.

References:

Hincks, M.J., McCannel, A. and Stanley, D.W. 1987. Hard-to-cook defect in black beans. Soaking and cooking processes. J. Agric. Food Chem. 35:576-583.

Hohlberg, A.I. and Stanley, D.W. 1987. Hard-to-cook defect in black beans. Protein and starch considerations. J. Agric. Food Chem. 35:571-576.

Plhak, L.C., Stanley, D.W., Hohlberg, A.I. and Aguilera, J.M. 1987. Hard-to-cook defect in black beans - effect of pretreatment and storage condition on extractable phenols and peroxidase activity. Can. Inst. Food Sci. Technol. J. 20(5):378-382.

Sefa-Dedeh, S., Stanley, D.W. and Voisey, P.W. 1978. Effects of soaking time and cooking conditions on texture and microstructure of cowpeas (Vigna unguiculata). J. Food Sci. 43:1832-1838.

Sefa-Dedeh, S., Stanley, D.W. and Voisey, P.W. 1979. Effect of storage time and conditions on the hard-to-cook defect in cowpeas (Vigna unguiculata). J. Food Sci. 44:790-796.

Voisey, P.W. and Larmond, E. 1971a. Report on the measurement of cooked soybean toughness. Engineering Research Service No. 230. Agriculture Canada, Research Branch, Ottawa.

Voisey, P.W. and Larmond, E. 1971b. Texture of baked beans-a comparison of several methods of measurement. J. Text. Studies 2:96-109.

Voisey, P.W. and Nonnecke, I.L. 1973. Measurement of pea tenderness. V: J. Text. Studies 4:323-343.

OTMS (INSTRON) PEAK FORCE MEASUREMENT OF RAW BEANS

Adapted from the method of Sefa-Dedah et al. 1978.

Purpose: Determination of the hardness of raw beans, expressed in newtons.

Scope: Applicable to all types of raw, unsoaked beans or beans soaked for varying lengths of time.

Limitations: Time consuming as only one bean can be tested at a time. Since there can be a large variation between beans, a large number of beans should be tested to obtain a representative sample mean.

Apparatus:

1. Ottawa Texture Measuring System (Canner's Machinery Ltd., Simcoe, Ontario) [or Instron Universal Texture Testing System (Instron Canada, Burlington, Ontario)]. Data are recorded using an Apple 2e computer, connected to the texture testing instrument.
2. Wedge apparatus consisting of the upper attachment of the OTMS bite test cell (Model D-1203, Canada Machinery Ltd., Simcoe, Ontario), a stainless steel wedge, the lower attachment of the OTMS compression cell fitted with a cross-slide and an aluminum plate test cell (fitted to the cross-slide) with a spherical recess.
4. Texture Data Acquisition and Analysis Computer Program (Agriculture Canada, 1986).

Sample Preparation:

Randomly select a sample of 30 beans.

Procedure:

The hardness of raw beans is measured on an Ottawa Texture Measuring System (OTMS) using a wedge apparatus and a 45.4 kg (100 lb) load cell. Test conditions include a crosshead speed of 6.6 cm/min, a test time of 30 sec and a transducer capacity of 45.4 kg.

Attach the signal conditioner to the OTMS. The signal conditioner should be turned on at least one half hour before use to allow it to warm up. Mount the load cell on the moving crosshead of the OTMS and attach the chuck and wedge. Calibrate the OTMS-Apple 2e system using weights.

Place the bean to be tested on its flat side, with the hilum facing the experimenter, in the spherical recess of the lower test cell.

Lower the stainless steel cutting wedge to cut the bean. To prevent the wedge from touching the plate, stop the downward movement when the wedge is 0.2 mm from the plate. Repeat with the remaining 29 beans.

Record the peak force required to cut through each bean and calculate an average peak force value over the 30 beans.

Notes:

Hohlberg and Stanley (1987) soaked beans for 18 hr at 25°C before testing the hardness of 20 beans.

Sefa-Dedeh et al. (1978, 1979) soaked cowpeas in water at 25°C for 1,3,6,12,18 and 24 hr before making texture measurements using a 500 kg load cell.

References:

Hohlberg, A.I. and Stanley, D.W. 1987. Hard-to-cook defect in black beans. Protein and starch considerations. J. Agric. Food Chem. 35:571-576.

Sefa-Dedeh, S., Stanley, D.W. and Voisey, P.W. 1978. Effects of soaking time and cooking conditions on texture and microstructure of cowpeas (Vigna unguiculata). J. Food Sci. 43:1832-1838.

Sefa-Dedeh, S., Stanley, D.W. and Voisey, P.W. 1979. Effect of storage time and conditions on the hard-to-cook defect in cowpeas (Vigna unguiculata). J. Food Sci. 44:790-796.

MATTSON BEAN COOKER RELATIVE COOKING TIME METHOD

Purpose: Determination of relative cooking times for beans.

Scope: Applicable to all types of raw or soaked beans.

Limitations: The cooking time does not represent the length of time needed to cook beans to the degree of doneness acceptable for human consumption.

Apparatus:

1. Mattson Bean Cooker (CIAT Model) with 25 - 90g plungers (rounded penetrating tips)
2. Analytical balance, accurate to 0.01g
3. Water bath with lid
4. Metal or plastic sieve
5. Household stove or heating unit
6. 2 L beaker or coffee pot (Pyrex model 7759)
7. 100 ml beakers, for soaking beans
7. Stop watch

Sample Preparation:

Weigh approximately 50 beans, rinse gently with warm water and soak in a 5:1 ratio of distilled water to dry bean weight for 24 hr, in a covered 25°C water bath.

Drain the soaked beans for 2 min in a sieve. Discard damaged or discoloured beans and beans with split skins.

Procedure:

Randomly choose 25 soaked beans and place them in the 25 indentations on the bottom plate of the bean cooker. Rest the plunger tips on the surface of the beans such that the plungers will fall through the center of the beans. Submerge the bean cooker with plungers and beans in 1030 ml of distilled water in a beaker or pyrex coffee pot. Cover the area between the top of the bean cooker and the top of the pot with a piece of aluminum foil, if necessary, to prevent excessive evaporation of the water. Quickly bring the water to a boil on high heat and then reduce the temperature to maintain a slow boil. Begin cooking time measurement when the water starts to boil. Maintain the water at a constant level throughout cooking by adding boiling water. The time required for thirteen of the 25 plungers (50%) to fall through their respective beans is recorded as the cooking time.

Replicate 2 times.

Notes:

The plunger weight and the type and diameter of the plunger tip may vary with the design of the cooker. By varying these two details, a cooker may be designed such that the determined cooking time, for a specific type of bean, will reflect a sensory panel's assessment of doneness (Proctor and Watts, 1987).

Recording the number of plungers falling within specified time intervals, such as 5 min intervals, allows the drawing of a cooking time curve. Cumulative totals of the plungers dropped can be plotted, as percent cooked, against cooking time to produce the curve.

Mattson (1946) originally designed the cooker for peas. It had 100 indentations for 100 peas and 82g plunger weights. The water level in the pot was kept 1 inch below the peas and the water boiled vigorously throughout the cooking period. Cooking was complete when all the plungers had fallen.

Proctor (1985) soaked navy beans in a 4:1 ratio of distilled water to beans for approximately 16 hr at 20°C. Plungers with flat-faced tips and weight/diameter combinations of 65g/2 mm, 37.5g/2 mm, 49.75g/5 mm and 48g/5 mm were used. The number of fallen plungers was recorded at 5 min intervals until all of the plungers had dropped or until 90 min had elapsed, whichever came first.

References:

Mattson, S. 1946. The cookability of yellow peas. A colloid-chemical and biochemical study. Acta. Agr. Suecana II 2:185-231.

Proctor, J.R. 1985. Navy bean cookability evaluation by modified Mattson bean cooker and by sensory panels. M.Sc. thesis, University of Manitoba, Winnipeg, Canada.

Proctor, J.R. and Watts, B.M. 1987. Development of a modified Mattson bean cooker procedure based on sensory panel cookability evaluation. Can. Inst. Food Sci. Technol. J. 20(1):9-14.

MOISTURE CONTENT OF BEANS

Based on AACC METHOD 44-15A (1983)

Purpose: Determination of the moisture content of fresh or stored beans.

Scope: Applicable to all types of beans.

Apparatus:

1. Wiley laboratory mill, equipped with 18- or 20-mesh screen and a 4-oz receiving bottle; or any other mill that will grind to the same degree of fineness without undue exposure to atmosphere and without appreciable heating.
2. Oven capable of being maintained at 130°C (Procedure 1) or 103°C (Procedure 2) uniformly throughout the oven, and provided with good ventilation.
3. Aluminum moisture dishes (approximately 55 mm diameter, 15 mm height) with slightly tapered sides and tightly fitting slip-in covers that fit snugly under dishes when placed in oven. Both dish and cover should be identified by the same number. Before using, dry for 1 hr at 130°C and cool in desiccator for 1 hr.
4. Airtight desiccator containing activated alumina, molecular sieves (type 4A or 4AXW) or other equally suitable desiccant.
5. Analytical balance, accurate to 0.01g
6. Plastic containers (225 ml)

Procedure:

1. Two-Stage Method

   For samples containing 16% or more moisture (10% or more for soybeans).

   Weigh plastic container, add approximately 50g beans and record exact weight. Leave the beans in an open container overnight or longer, (14-60 hr depending on the temperature) in a well-ventilated place (preferably on top of heated oven protected from dust) to air dry. Moisture content should be reduced to less than 16%. Weigh beans and container, after air drying, and record weight.

   Grind beans in a Wiley mill using a 20 mesh screen and gravity feed. Do not push the beans into the mill with a plunger or overheating may occur. Catch the ground beans in an 225 ml glass bottle. Seal after grinding. Shake bottle well to mix contents.

Remove moisture dishes from the desiccator, one at a time. Weigh moisture dish, add approximately 2g of well mixed ground beans and record exact weight. Place uncovered moisture dishes, with lids under dishes, in a 130°C oven for exactly 60 minutes after oven recovers its temperature. (Oven should regain temperature within 15-20 min after insertion of a full load of 24 dishes).

Immediately cover moisture dishes (using finger insulators) and transfer dishes to a desiccator as quickly as possible. Allow dishes to cool to room temperature (45-60 min) and record weight.

Calculate total percent moisture using calculations 1, 2 and 3 below.

2. One-Stage Method for Corn and Beans (103°C)

For samples containing less than 16% moisture except soybeans which should be under 10%.

Remove moisture dishes from the desiccator, one at a time. Weigh moisture dish, add approximately 15g of beans and record exact weight. Place uncovered moisture dishes, with lids under dishes, in a 103°C oven for 72 hr.

Immediately after heating, cover moisture dishes using finger insulators and transfer dishes to a desiccator as quickly as possible. Allow dishes to cool to room temperature (45-60 min) and record weight.

Calculate total percent moisture using calculation 2.

Calculations:

1. First stage:

$$\underset{[A]}{\%\ \text{Moisture}} = 1 - \left[\frac{\text{bean wt after air drying}}{\text{bean wt before air drying}}\right] \times 100$$

2. Second stage:

$$\underset{[B]}{\%\ \text{Moisture}} = 1 - \left[\frac{\text{bean wt after oven drying}}{\text{bean wt before oven drying}}\right] \times 100$$

3. $$\text{Total \% Moisture} = A + \frac{(100-A)B}{100}$$

Precision:

Replicate determinations should check within 0.2% moisture.

Reference:

AACC 1983. AACC Method 44-15A, Moisture-air-oven methods. In 'Approved Methods of the American Association of Cereal Chemists,' Eighth Edition, American Association of Cereal Chemists, Inc., St. Paul, MN.

WHOLE BEAN AND COTYLEDON WATER ABSORPTION

Purpose: Determination of water absorption capacity of whole beans and bean cotyledons.

Apparatus:

1. Analytical balance, accurate to 0.01g
2. 100 ml beakers. Before using, dry for 24 hr at 60°C and cool in desiccator for 1 hr
3. Water bath with lid
4. Two 500 ml glass jars, that have been half-filled with glass beads
5. Two watch glasses, large enough to cover the tops of the glass jars
6. 50 ml beakers, or other suitable containers which will fit inside the jars
7. Oven capable of being maintained at 60°C
8. Aluminum moisture dishes. Before using, dry for 24 hr at 60°C and cool in desiccator for 1 hr
9. Desiccator containing chemical desiccant
10. Nylon netting
11. Scalpel

Sample Preparation:

Determine the moisture content of the beans using the methodology previously described.

Randomly select a sample of 15 beans.

Procedure:

Remove 100 ml beaker from desiccator and weigh.

Weigh the 15 beans ('as is' wt) and place them in a 15 cm square of nylon netting. Bring the corners of the netting together and place the netting and beans into the weighed beaker. Add distilled water to the beaker to cover the beans (10:1 ratio of water to 'as is' bean weight). Set the beaker into a covered 25°C water bath and soak for 24 hrs. (When preparing more than 1 sample, stagger the soaking times by 15 min intervals).

Add water to the top of the glass beads in each glass jar and set a 50 ml beaker on the beads. Weigh one of the glass jars with contents and watch glass (W1).

Lift the nylon netting and soaked beans out of the soaking water and rinse the beans with distilled water, collecting the rinse water in the beaker containing the soaking water. Drain

the soaked beans for 2 min in the netting, over the soaking beaker, and blot dry with Kimwipe tissues for 10 sec to remove surface moisture. Immediately put the soaked beans into the 50 ml beaker inside the weighed glass jar, cover the jar and weigh (W2).

Weigh two moisture dishes and record their numbers and weights.

Weigh the second glass jar with contents and watch glass (W3). Take the beans from the first jar, one at a time replacing the lid after each removal, and quickly remove the seedcoat with a scalpel. Place the seedcoats in one of the weighed aluminum weighing dish. Put the cotyledons of the beans into the 50 ml beaker of the second jar and weigh (W4). Place the weighed cotyledons into the second weighing dish.

Dry seedcoats and peeled cotyledons to a constant weight (24 hr) in a 60°C oven. Place the beaker of soaking/rinsing water in the 60°C drying oven to evaporate the moisture and dry to constant weight. After drying, cool the samples to room temperature in a desiccator for 1 hr. Weigh dried seedcoats to obtain seedcoat dry weight, weigh dried peeled cotyledons to obtain soaked cotyledon dry weight and weigh beaker plus residue to obtain leached solids weight.

Do duplicates of all determinations.

Calculations:

W2 - W1 = HYDRATED WT

W4 - W3 = HYDRATED COTYLEDON WT

$$\text{ORIGINAL SOLIDS WT} = \text{'AS IS' WT} - \frac{\%\ \text{MOISTURE}}{100} \times \text{'AS IS' WT}$$

The following can be calculated:

1. WHOLE BEAN WATER CONTENT AS PERCENT OF SOLIDS

$$\frac{(\text{HYDRATED WT} - \text{ORIGINAL SOLIDS WT})}{\text{ORIGINAL SOLIDS WT}} \times 100$$

2. WHOLE BEAN WATER CONTENT AS PERCENT OF SOLIDS REMAINING AFTER SOAKING (CORRECTED FOR SOLIDS LOST)

$$\frac{(\text{HYDRATED WT} - \text{ORIGINAL SOLIDS WT} + \text{LEACHED SOLIDS WT})}{(\text{ORIGINAL SOLIDS WT} - \text{LEACHED SOLIDS WT})} \times 100$$

3. COTYLEDON (COTYL) WATER CONTENT AS PERCENT OF WHOLE BEAN SOLIDS

$$\frac{\text{(HYDRATED COTYL WT - COTYL SOLIDS WT AFTER SOAKING)}}{\text{ORIGINAL WHOLE BEAN SOLIDS WT}} \times 100$$

4. COTYLEDON WATER CONTENT AS PERCENT OF ORIGINAL COTYLEDON SOLIDS

$$\frac{\text{(HYDRATED COTYL WT - COTYL SOLIDS WT AFTER SOAKING)}}{\text{(COTYL SOLIDS WT AFTER SOAKING + LEACHED SOLIDS WT)}} \times 100$$

PROXIMATE COMPOSITION OF BEANS

FAT CONTENT OF BEANS

Adapted from AACC Method 30-25 (1983)

Purpose: Determination of the fat content of beans.

Procedure Modifications:

Air dry and grind a sample of beans. Extract 6g of ground sample in a soxhlet for 6 hr with hexane.

Reference:

AACC 1983. AACC Method 30-25, Crude fat in wheat and soy flour, feeds and cooked feeds. In 'Approved Methods of the American Association of Cereal Chemists,' Eighth Edition, American Association of Cereal Chemists, Inc., St. Paul, MN.

PROTEIN CONTENT OF BEANS

Adapted from AACC Method 46-12 (1983)

Purpose: Determination of the protein content of beans.

Procedure Modifications:

Air dry and grind a sample of beans. Determine the proteins in a 300 mg sample of ground beans by macro-kjeldahl, using titanium dioxide as a catalyst. Use a conversion factor from nitrogen to protein of 6.25.

Notes:

Williams et al. (1988) described a near-infrared reflectance method for protein determination of legumes.

Reference:

AACC 1983. AACC Method 46-12, Crude protein-Kjeldahl method, boric acid modification. In 'Approved Methods of the American Association of Cereal Chemists,' Eighth Edition, American Association of Cereal Chemists, Inc., St. Paul, MN.

Williams, P., El-Haramein, F.J., Nakkoul, H. and Rihawi, S. 1988. 'Crop Quality Evaluation Methods and Guidelines.' International Center for Agricultural Research in the Dry Areas (ICARDA), Aleppo, Syria.

ASH DETERMINATION OF BEANS

AACC Method 08-03 (1983)

Purpose: Determination of the ash content of beans as the residue remaining after incineration.

Reference:

AACC 1983. AACC Method 08-03, Ash-rapid (2-hour, 600o) method. In 'Approved Methods of the American Association of Cereal Chemists,' Eighth Edition, American Association of Cereal Chemists, Inc., St. Paul, MN.

PHYTIC ACID

Modification of the method of Latta and Eskin (1980)

Purpose: The extraction and measurement of phytate in beans.

Scope: Applicable to a variety of legumes, cereals and oilseeds.

Apparatus:

1. Analytical balance, accurate to 0.01g
2. 20 x 125 mm screw top tubes
3. Water bath shaker
4. Centrifuge tubes
5. Centrifuge
6. Vials
7. 0.7 cm x 27 cm ion exchange column filled with 0.4g of 200-400 mesh AG1-X8 chloride anion exchange resin (Biorad)
8. 25 ml Volumetric flask
9. Pipets
10. 15 ml Conical tubes
11. Parafilm
12. Spectrophotometer (SP6-300)

Reagents:

0.65 N Hydrochloric acid
1.4 N Hydrochloric acid
0.1 N Sodium chloride
0.7 N Sodium chloride
Wade reagent
Stock standard - 200 ug phytic acid/ml

Sample Preparation:

Prepare working standards containing 10, 20, 30 and 40 ug phytic acid/ml by diluting 5, 10, 15 and 20 ml stock standard respectively to 100 ml with 0.7 N NaCl.

Determine the moisture content of the beans using the methodology previously described.

Procedure:

Weigh 1.00g sample and transfer to a 20 x 125 mm screw top tube. Add 20 ml 0.65 N HCl and cap tightly. Place tubes horizontally in a water bath shaker and shake for 1 hr. Centrifuge tubes at full speed for 10 min and transfer 5-10 ml supernatant to a vial and store in fridge.

Elute column with 5 ml of 1.4 N HCl. Elute column with 10 ml of deionized water. Dilute sample 5/25 and pipet 2 ml onto

the column. After the sample has passed through the column add 10 ml of 0.1 N NaCl to the column to elute inorganic phosphorous and impurities. Once this has passed through, discard the eluant and place 15 ml conical tubes under the column. Elute the phytate from the column with exactly 10 ml 0.7 N NaCl.

While the sample is eluting, prepare standards by pipetting 10 ml into conical tubes. The standards must be at room temperature. Do 2 blanks and one of each of the standards. (The blank consists of 0.7 N NaCl).

When the sample has eluted add 3.0 ml Wade reagent to blanks, standards and samples. Cover tubes with parafilm and invert to mix. Centrifuge at 3/4 speed for 5 min to precipitate the phytic acid.

Read absorbance of all tubes at 500 nm using deionized water to zero the instrument. Subtract absorbance readings for standards and sample from the average absorbance reading for the blanks to obtain final absorbance readings.

Plot absorbance readings for the standards against concentration of the added standards to obtain a standard curve. Apply final absorbance readings of the samples to the standard curve to determine the concentration of the samples in ug/ml. Divide the answer in ug/ml by a factor of 20 to obtain an answer in % phytic acid in the original sample. Report % phytic acid on a dry weight basis.

Reference:

Latta, M. and Eskin, M. 1980. A simple and rapid colorimetric method for phytate determination. J. Agric. Food Chem. 28:1313-1315.

SEED WEIGHT

Adapted from the method of Elias, Garcia-Soto and Bressani (1986)

Purpose: Determination of the average weight of a randomly selected sample of beans.

Scope: Applicable to all types of beans.

Apparatus:

Analytical balance, accurate to 0.01g

Sample Preparation:

Determine the moisture content of the beans using the methodology previously described.

Randomly select a sample of approximately 100 beans. Exclude broken seeds and other foreign material.

Procedure:

Weigh the bean sample. Manually count the number of beans in the sample.

Replicate 3 times.

Calculate mean weight and standard deviation for the 3 replications (ie. g/100 beans).

Report mean weight, standard deviation and moisture content of the bean sample.

Notes:

Reference values - Average weights of common black beans

<0.193g	= small size seed
0.193-0.217g	= medium size seed
>0.217g	= large size seed

Individual weights of 25 beans give more information about variability within the sample than the weight from a group of 25 beans.

Reference:

Elias, L.G., Garcia-Soto, A. and Bressani, R. 1986. Metodos para establecer la calidad tecnologica y nutricional del frijol. Institute of Nutrition of Central America and Panama (INCAP), Guatemala, Central America.

SEED SIZE DISTRIBUTION

Purpose: Determination of the size distribution of a sample of beans.

Scope: Applicable for raw and soaked beans of all types.

Apparatus:

1. Analytical balance, accurate to 0.01g
2. Set of 6 sieves that nest together (The Clipper Grain Seed and Bean Cleaners, Manufactured by A.T. Ferrel & Co., Saginaw, MI.), each 13 inches in diameter with oblong slots (parallel sides and semi-circular ends) 3/4 inch long and ranging in width from 9/64 to 14/64 inches, in increments of 1/64 of an inch.
3. Mechanical shaking device for the sieves.

Sample Preparation:

Randomly select and weigh a representative 1 kg sample of beans.

Procedure:

Arrange the sieves from largest to smallest slot size with the sieve with the smallest slots at the bottom. Transfer the 1 kg sample of beans to the top sieve and pass it through the set of sieves by shaking on a mechanical shaking device until beans are sorted by sieve size.

Place the beans remaining on each of the six sieves and in the bottom of the pan in labelled containers and weigh each container of beans.

Calculate the percentage of the original weight of beans represented by the beans retained on each sieve size.

Repeat the test using a second 1 kg sample and average the results of the two tests.

Notes:

Sereda (1989) used 7 kg of beans, and Bourne (1967) used a 100 lb sample of beans to obtain the size distribution.

Dos Santos Garruti and Bourne (1985) and Rozo (1982) used sets of wooden sieves, similar to those described above to size grade red kidney beans. The fraction of beans passing through 17/64 inch slots and retained by 12/64 inch slots (95% of the beans) was used in their experiments.

Hulse et al.(1977) reported the use of a set of wooden-frame sieves with slot sizes of 20/64 to 8/64 inch by 1/64 inch increments with 100 lb and 3 kg sample sizes to size grade recently harvested beans and beans soaked for 18 hr.

References:

Bourne, M.C. 1967. Size, density and hardshell in dry beans. Food Technol. 21:335-338.

dos Santos Garruti, R. and Bourne, M.C. 1985. Effect of storage conditions of dry bean seeds (Phaseolus vulgaris L.) on texture profile parameters after cooking. J. Food Sci. 50:1067-1071.

Hulse, J.H., Rachie, K.O. and Billingsley, L.W. (Ed.) 1977. 'Standards and Methods of Evaluation for Food Legume Breeders.' IDRC-Ts7e, Ottawa, Canada.

Rozo, C. 1982. Effect of extended storage on the degree of thermal softening during cooking, cell wall components, and polyphenolic compounds of red kidney beans (Phaseolus vulgaris). Ph.D. thesis, Cornell University, Ithaca, N.Y.

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PERCENT SEEDCOAT 1

Method of Elias, Garcia-Soto and Bressani (1986)

Purpose: Determination of the relationship between the weight of the dry seedcoats of 25 beans and the weight of dry cotyledons plus seedcoats, expressed as a percentage.

Scope: Applicable to all types of beans.

Limitations: Does not take into consideration the solids lost, from cotyledons and seedcoats, during 16-18 hr of soaking.

Apparatus:

1. Analytical balance, accurate to 0.01g
2. Vacuum oven, calibrated to 60°C and a pressure of 25mm Hg
3. Desiccator containing chemical desiccant

Sample Preparation:

Randomly select a sample of 25 beans.

Procedure:

Soak the sample of 25 beans overnight (16-18 hr) in 50 ml of room temperature distilled water. Dry the soaked beans with a paper towel and manually separate the seedcoat from the cotyledon of each bean. Dry the seedcoats and cotyledons in a 60°C vacuum oven for 4 hr. Cool the dried seedcoats and cotyledons to room temperature in a desiccator and weigh.

Replicate 3 times.

Calculations:

$$\% \text{ Seedcoat} = \frac{\text{seedcoat dry wt}}{\text{cotyledon dry wt + seedcoat dry wt}} \times 100$$

Notes:

Reference values - < 8% = low seedcoat content
8-10% = medium seedcoat content
> 10% = high seedcoat content

To adjust for leached solids, dry beaker containing soaking water in 60°C vacuum oven to constant weight. The calculation, adjusting for leached solids is:

% Seedcoat (adjusted for leached solids) =

$$\frac{\text{seedcoat dry wt}}{\text{cotyledon dry wt + seedcoat dry wt + leached solids dry wt}} \times 100$$

Reference:

Elias, L.G., Garcia-Soto, A. and Bressani, R. 1986. Metodos para establecer la calidad tecnologica y nutricional del frijol. Institute of Nutrition of Central America and Panama (INCAP), Guatemala, Central America.

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[1] Alternatively, percent seedcoat may be determined as part of the whole bean and cotyledon water absorption methodology, as previously described.

SEEDCOAT THICKNESS

Adapted from the method of Lareo (1986)

Purpose: Estimation of the seedcoat thickness of a sample of beans using the mean length, depth, width, seed weight and percent seedcoat measurements of the sample.

Scope: Applicable to all types of beans.

Apparatus:

1. Vernier calipers

Sample Preparation:

Randomly select a representative sample of 30 beans.

Procedure:

Measure the length, depth and width of 30 beans in centimeters (2 decimals) using vernier calipers. Calculate the mean measurements.

Determine the mean seed weight and mean percent seedcoat as in previously described methods.

Calculations:

$$\text{Thickness} = \frac{\text{seed weight (mg) x \% seedcoat /100}}{\text{surface area (cm}^2\text{)}} = \text{mg/cm2}$$

where: $\text{surface area} = 2\pi A2 + \pi \left[\frac{B2}{E} \ln \frac{(1+E)}{(1-E)}\right]$

where: $A = \frac{a}{2}$ a = length (cm) -- see Figure 1

$B = \frac{c + b}{4}$ b = depth (cm) -- see Figure 1
c = width (cm) -- see Figure 1

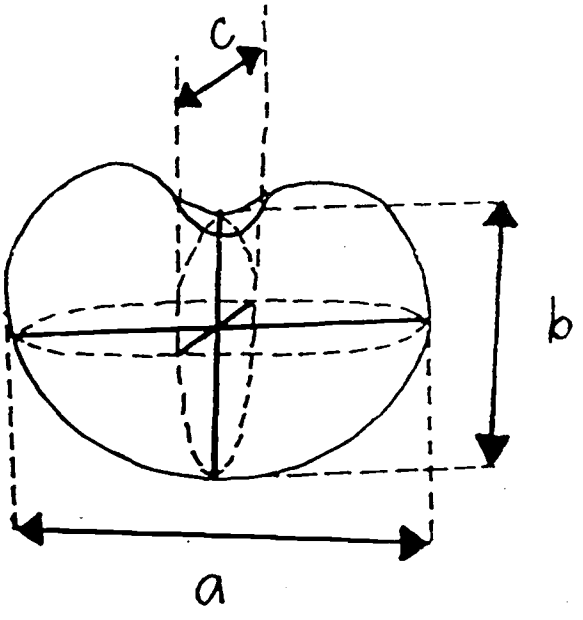
$E = \frac{\sqrt{A2 - B2}}{A}$

Notes:

Lareo (1986) recommends the use of a nonium or micrometer to obtain measurements in micrometers.

Reference:

Lareo, L. 1986. Personal Communication. CIAT, Columbia, South America.



a: Length (cm)
b: Depth (cm)
c: Width (cm)

Figure 1: Basic size dimensions of a bean measured for use in seedcoat thickness calculations (Sereda, 1989)

Sereda, L.M. 1989. Comparison of the cookability and texture characteristics of six lines of Guatemalan bush and vine black beans (Phaseolus vulgaris) - as determined by trained and untrained sensory panels and the Ottawa texture measuring system. M.Sc. thesis, University of Manitoba, Winnipeg, Canada.

PERCENT HARDSHELL

Purpose: Determination of the number of hardshell beans in a sample of beans.

Scope: Applicable to all types of beans.

Apparatus:

1. 250 ml beakers
2. Analytical balance, accurate to 0.01g
3. Metal or plastic strainer

Sample Preparation:

Randomly select approximately 50g of beans. Count the number of beans in the sample.

Procedure:

Place the beans in a 250 ml beaker containing 150 ml distilled water and soak for 16 hr at room temperature (20°C). At the end of the soaking period, drain the beans and count the number of hardshell beans or seeds that did not imbibe water (did not swell) during the soaking period.

Calculate the total number of hardshell beans as a percent of the original number soaked.

Calculations:

$$\% \text{ Hardshell} = \frac{\text{no. hardshell beans}}{\text{total no. beans soaked}} \times 100$$

Notes:

Williams et al.(1988) report the use of 100 beans (chickpeas and lentils) or 25-50 beans (faba beans) depending on the size. Beans were soaked for 16 hr at room temp (22-25°C).

Reference:

Williams, P., El-Haramein, F.J., Nakkoul, H. and Rihawi, S. 1988. 'Crop Quality Evaluation Methods and Guidelines.' International Center for Agricultural Research in the Dry Areas (ICARDA), Aleppo, Syria.